

Diminished serum repetin levels in patients with schizophrenia and bipolar disorder

By: Shuai Wang, Huixun Ren, Jie Xu, Yanjun Yu, Shuiping Han, Hui Qiao, Shaoli Cheng, Chang Xu, Shucheng An, Bomiao Ju, Chengyuan Yu, Chanyuan Wang, Tao Wang, Zhenjun Yang, [Ethan Will Taylor](#), and Lijun Zhao

Shuai Wang, Huixun Ren, Jie Xu, Yanjun Yu, Shuiping Han, Hui Qiao, Shaoli Cheng, Chang Xu, Shucheng An, Bomiao Ju, Chengyuan Yu, Chanyuan Wang, Tao Wang, Zhenjun Yang, Ethan Will Taylor & Lijun Zhao. Diminished serum repetin levels in patients with schizophrenia and bipolar disorder. *Scientific Reports* 5, 7977; <https://doi.org/10.1038/srep07977> (2015).

Published under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (CC BY-NC-SA 4.0); <http://creativecommons.org/licenses/by-nc-sa/4.0/>

Abstract:

Repetin (RPTN) protein is a member of S100 family and is known to be expressed in the normal epidermis. Here we show that RPTN is ubiquitously expressed in both mouse and human brain, with relatively high levels in choroid plexus, hippocampus and prefrontal cortex. To investigate the expression of RPTN in neuropsychiatric disorders, we determined serum levels of RPTN in patients with schizophrenia (n = 88) or bipolar disorder (n = 34) and in chronic psychostimulant users (n = 91). We also studied its expression in a mouse model of chronic unpredictable mild stress (CUMS). The results showed that serum RPTN levels were significantly diminished in patients with schizophrenia and bipolar disorder or in psychostimulant users, compared with healthy subjects (n = 115) or age-matched controls (n = 92) ($p < 0.0001$). In CUMS mice, RPTN expression in hippocampus and prefrontal cortex was reduced with progression of the CUMS procedure; the serum RPTN level remained unchanged. Since CUMS is a model for depression and methamphetamine (METH) abuse induced psychosis recapitulates many of the psychotic symptoms of schizophrenia, the results from this study may imply that RPTN plays a potential role in emotional and cognitive processing; its decrease in serum may indicate its involvement in the pathogenesis of schizophrenia and bipolar disorder.

Keywords: schizophrenia | bipolar disorder | hippocampus | repetin expression

Article:

*****Note: Full text of article below**



OPEN

SUBJECT AREAS:
SCHIZOPHRENIA
HIPPOCAMPUSReceived
3 July 2014Accepted
23 December 2014Published
23 January 2015Correspondence and
requests for materials
should be addressed to
L.J.Z. (zhaolijun@snnu.
edu.cn)* These authors
contributed equally to
this work.Diminished serum repetin levels in
patients with schizophrenia and bipolar
disorderShuai Wang^{1*}, Huixun Ren^{2*}, Jie Xu¹, Yanjun Yu³, Shuiping Han⁴, Hui Qiao⁵, Shaoli Cheng⁶, Chang Xu⁵,
Shucheng An⁵, Bomiao Ju¹, Chengyuan Yu¹, Chanyuan Wang¹, Tao Wang¹, Zhenjun Yang⁷,
Ethan Will Taylor⁸ & Lijun Zhao¹

¹Key Laboratory of Ministry of Education for Medicinal Plant Resource and Natural Pharmaceutical Chemistry, College of Life Sciences, Shaanxi Normal University, Xi'an 710062, China, ²Department of Pathogenic Biology and Immunology, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, China, ³Department of Clinical Chemistry, Xi'an mental health center, Xi'an 710061, China, ⁴Department of Pathology, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, China, ⁵College of Life Sciences, Shaanxi Normal University, Xi'an 710062, China, ⁶Center for Experimental Morphology, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, China, ⁷The State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100083, China, ⁸Department of Nanoscience, Joint School of Nanoscience and Nanoengineering, University of North Carolina at Greensboro, Greensboro, NC 27401, USA.

Repetin (RPTN) protein is a member of S100 family and is known to be expressed in the normal epidermis. Here we show that RPTN is ubiquitously expressed in both mouse and human brain, with relatively high levels in choroid plexus, hippocampus and prefrontal cortex. To investigate the expression of RPTN in neuropsychiatric disorders, we determined serum levels of RPTN in patients with schizophrenia (n = 88) or bipolar disorder (n = 34) and in chronic psychostimulant users (n = 91). We also studied its expression in a mouse model of chronic unpredictable mild stress (CUMS). The results showed that serum RPTN levels were significantly diminished in patients with schizophrenia and bipolar disorder or in psychostimulant users, compared with healthy subjects (n = 115) or age-matched controls (n = 92) (p < 0.0001). In CUMS mice, RPTN expression in hippocampus and prefrontal cortex was reduced with progression of the CUMS procedure; the serum RPTN level remained unchanged. Since CUMS is a model for depression and methamphetamine (METH) abuse induced psychosis recapitulates many of the psychotic symptoms of schizophrenia, the results from this study may imply that RPTN plays a potential role in emotional and cognitive processing; its decrease in serum may indicate its involvement in the pathogenesis of schizophrenia and bipolar disorder.

Schizophrenia and bipolar disorder are considered to be “multiple hit” diseases, stemming from genetic and environmental influences^{1,2}. Although the etiology of these diseases is largely unknown, there is growing evidence that dysregulation of calcium signaling is involved^{3,4}. Calcium-binding proteins are mediators of a variety of cellular processes including calcium signaling. Parvalbumin (PV) is a calcium binding protein that is frequently reduced in PV basket cells in the prefrontal cortex and hippocampus of schizophrenic subjects^{5,6}. Decreased densities of PV-positive interneurons in the para- and hippocampal regions was also observed in both bipolar disorder and schizophrenia^{7,8}, indicating hippocampal dysfunction in those disorders and a potential key role for PV in the pathophysiology of schizophrenia⁶. Calcium binding protein S100B is abundant in CNS and has been suggested to be a susceptibility gene for bipolar disorder, schizophrenia and cognitive dysfunction⁹. In bipolar patients, the densities of S100B-immunopositive astrocytes in the CA1 pyramidal layer and S100B-immunopositive oligodendrocytes in the left alveus are decreased¹⁰. In schizophrenia, S100B is increased in the cerebrospinal fluid (CSF), indicating glial cell dysfunction¹¹. Whether an elevated serum level of S100B is a marker for psychotic illness still remains controversial. Some studies have suggested that S100B is an indicator of astrocyte activation and brain dysfunction^{12–14}; in contrast, others have claimed that it is neither an intermediate phenotype nor a trait marker¹⁵.

The human repetin (RPTN) protein is a new member of the S100 family with two EF-hand domains in the N-terminal that can reversibly bind calcium. RPTN was originally identified as a member of the “fused” gene family



that is associated with keratin intermediate filaments¹⁶. RPTN expression was found in normal epidermis but is also high in the acrosyngium, the inner hair root sheath and in the filiform papilli of the tongue¹⁷.

Until recently, RPTN was not known to have any function in the nervous system, or even to be expressed in the brain. In this study, we examined its expression in the CNS and in sera from patients with schizophrenia and bipolar disorder, and from psychostimulant users. We also employed the chronic unpredictable mild stress (CUMS)¹⁸ mouse model to investigate whether RPTN is potentially involved in the development of emotional and cognitive dysfunctions.

Methods

Typical antipsychotics. Olanzapine was obtained from Jiangsu Hansoh pharmaceutical Co. and dissolved in 0.1 M HCl, pH-adjusted to 5.5 using 0.1 M NaOH and diluted with PBS to a final concentration of 0.025 mg/ml. Quetiapine fumarate was purchased from Hunan Dongting pharmaceutical Co., Ltd. and dissolved in PBS at a final concentration of 0.75 mg/ml. Aripiprazole was purchased from Shanghai Zhongxi pharmaceuticals and dissolved in PBS containing 5% DMSO.

Antibodies and western blot kit. Rabbit anti-human RPTN polyclonal antibody (cat. LS-B17) was purchased from LifeSpan BioSciences, Inc., USA. The antibody reacts with both human and mouse RPTN. Anti-β-actin (CB10099M) was purchased from California bioscience, USA; Goat anti-Rabbit IgG-HRP was purchased from Santa Cruz Biotech (cat. SC-2004). The SuperSignal West Pico kit (cat. 34079) was purchased from Thermo Scientific, USA.

Normal human brain chip. A normal human brain chip was purchased from US Biomax, Inc (cat. BNC17011). The chip is a normal brain tissue microarray of 26 cases/80 cores, containing three cases each of frontal lobe, apical lobe, occipital lobe, temporal lobe, midbrain, pons, medulla oblongata, thalamus opticus, cerebellum, hippocampus, callositas, optic nerve and spinal cord tissue, plus one case of caudate nucleus, with duplicate cores in each block.

Animals. Mouse CUMS model. The animal protocol of this study was approved by national legislations of China and local guidelines. The investigation was conducted in accordance with the ethical principles of animal use and care. 27 six-week old male BALB/c mice of 20–24 g weight were obtained from the animal center of Xi'an Jiaotong University and divided into two groups. Unless otherwise specified, mice were housed under a 12-hour light/12-hour dark cycle with free access to water and standard mouse diet (66% carbohydrate, 12% fat, 22% protein). 11 mice were maintained as control group, 16 mice were housed in individual cages and assigned as CUMS group. The CUMS mouse model was developed following the reported procedure¹⁸. Briefly, nine types of mild stressors (cage tilting, light–dark cycle, swimming in 4°C cold water, swimming in 45°C warm water, water deprivation, food deprivation, tail nip, shaking, and damp sawdust) were arranged randomly over a 21-day period and employed to stress mice. Mice were exposed to the stressors individually. Stressors were never performed simultaneously. Mice in the control group were not subjected to any stressors. The CUMS procedure used in this study has been employed successfully in our lab in both rats and mice¹⁹. Because the tail suspension test (TST) is widely recognized as a useful experimental paradigm for assessing depression-like behavior^{20,21}, TST was performed after 21 days of CUMS procedure to validate the model. Briefly, each mouse was suspended individually by its tail using adhesive laboratory tape to a flat metal bar connected to a strain gauge within a tail suspension chamber, Tail Suspension Monitor (TSE, German). The duration of the test was 6 min. Data acquisition and analysis was performed automatically. After collecting the blood samples, the brains from control and CUMS

mice were first perfused with 4% paraformaldehyde in PBS and then embedded in paraffin. Five-μm sagittal sections of mouse brain were prepared for immunohistochemical examination.

Immunohistochemistry. To investigate the expression pattern of RPTN in brain, mouse brain and human brain chips were treated with Rabbit anti-RPTN antibody (LS-B17). Briefly, after dewaxing and rehydration, sections were blocked with 20% blocking serum in TBS/0.1% Tween. Sections were then incubated with Rabbit anti-RPTN primary antibody (2.5 μg/ml) overnight at 4°C. The bound primary antibodies were detected using an EnVision + Dual Link System-HRP (K4063, Dako, USA). Slides were counterstained with hematoxylin.

Western blotting for RPTN alteration in CUMS mouse brain. To investigate alterations in RPTN expression during the CUMS procedure, 16 six-week old male BALB/c mice of 20–24 g weight were used in CUMS modeling as described above. Hippocampus and prefrontal lobes from four mice were removed on days 0, 7, 14 and 21. The tissue homogenates were freshly prepared by sonication in lysis buffer (50 mM Tris-HCl, 2 mM EDTA, 2 mM PMSF, pH 7.4) in an ice bath. Following homogenization, the tissue preparation was centrifuged for 2 minutes at 13,000 × g to collect the supernatant. The supernatants from each time point were stored at –80°C and used to run Western blots at the end of the CUMS procedure. Briefly, 40 μg of total protein from each mouse preparation of hippocampus or prefrontal cortex was applied on 10% SDS-PAGE gels for electrophoresis. After transferring onto PVDF membrane, the membrane was blotted with Rabbit anti-mouse RPTN antibody overnight at 4°C. After washing three times with PBS containing 0.05% of Tween-20, the membranes were incubated with goat anti-Rabbit IgG-HRP for one hour at room temperature. Finally, SuperSignal West Pico substrate was applied and images were obtained by x-ray film exposure. β-actin was used as an endogenous control. The blotting procedure was repeated on four mice from days 0, 7, 14 and 21. Densitometry analysis was used to measure the level of RPTN relative to β-actin in each sample and expressed as arbitrary units (a.u.). The alteration of RPTN in hippocampus or prefrontal lobe was statistically analyzed, with day zero animals used as a normal control.

Serum samples from human subjects. Healthy control sera (n = 115) were obtained from the physical examination center at the School of Medicine Xi'an Jiaotong University, Xi'an, China. Sera from patients with schizophrenia (n = 88) or bipolar disorder (n = 34) and from drug users (n = 91) were collected from Xi'an mental health center, Xi'an, China. Patients with schizophrenia or bipolar disorder were diagnosed by two psychiatrists according to ICD-10 criteria. All patients were inpatients under antipsychotic therapy at the time that blood samples were collected. The great majority of the patients were receiving atypical antipsychotics and mood stabilizers. Olanzapine, Quetiapine and Aripiprazole were mostly used by the patients. Drug users were enrolled from the methadone maintenance treatment program of Xi'an mental health center. All drug users had at least a three-year history of poly-drug (methamphetamine (METH) and heroin) abuse. There was no significant difference between healthy controls and patients in sex or age distribution, but drug users were predominantly males (Table 1).

Serum samples from CUMS mice and Mice treated with atypical antipsychotics. 36 six-week old male BALB/c mice of 20–24 g weight were obtained from the Animal Center of Xi'an Jiaotong University. Animals were divided into four groups and administered intraperitoneally with atypical antipsychotics Olanzapine (n = 9), Quetiapine (n = 7), Aripiprazole (n = 9) or PBS as control (n = 11) for 24 days. The dosages for Olanzapine, Quetiapine and Aripiprazole were 0.5 mg/kg/day²², 15 mg/kg/day²³ and 5 mg/kg/day²⁴, respectively. Sera were collected on day 25 for ELISA assays.

ELISA assay. Human and mouse RPTN ELISA kits (cat. CSB-EL020501HU, CSB-EL02051MO) were purchased from CUSABIO, Inc., Wuhan, China, with 156 and

Table 1 | Demographics of serum samples from healthy controls and patients

	Control	Schizophrenia	Bipolar Disorder	Drug user
Gender	58 M, 57 F	44 M, 44 F	M 18, 16 F	84 M, 7 F
Age	40.77 ± 13.26	38.87 ± 15.33	36.65 ± 15.07	47.5 ± 6.62
Duration of illness (years)	-	11.30 ± 10.1	10.06 ± 6.39	>3
Methadone-maintained	-	-	-	91
SANS	-	31.37 ± 22.99#	n.a	-
SAPS	-	21.09 ± 12.50#	n.a	-
Adjunct therapies (Bio-electric etc)	-	69	30	-
Typical Antipsychotics	-	3	1	-
Atypical	-	85	33	-
Antipsychotics	-	-	-	-
Antipsychotics plus mood stabilizer	-	40	34	-

M: male; F: female; n.a.: not available or a few were recorded; #: the latest scores.



78 pg/ml of the minimum detectable dose of RPTN, respectively. RPTN concentrations in collected serum samples from human subjects and mice were determined according to the manufacturer's instructions.

Statistical analysis. Kruskal-Wallis test was run in comparisons among multiple groups of humans or animals being compared. A two-tailed unpaired Mann-Whitney test was used in comparing two-group studies. One-way ANOVA followed by post-hoc Dunnett's test was run in comparisons among RPTN Western Blots.

Results

Expression of RPTN in human and mouse brain. RPTN was found to be ubiquitously expressed in human brain, with relatively high levels in choroid plexus (ependymal cells), hippocampus and prefrontal lobe (Figure 1,a,b,c) and weak staining in the temporal lobe (Figure 1,d). In normal mouse brain, strong RPTN-immunopositive staining is seen in locus ceruleus (LC), choroid plexus (ependyma), hippocampal CA3 and CA2 pyramidal layer and prefrontal cortex (Figure 2-a,b,c). Subcellularly, RPTN is expressed as puncta in the cytoplasm (Figure 1, b,c, inserts).

Decreased expression of RPTN in hippocampus and prefrontal lobe of CUMS mice. The TST test showed CUMS mice had significantly longer periods of immobility compared to normal animals (284.4 ± 2.14 sec/6 min vs. 241.9 ± 9.05 sec/6 min, $p < 0.001$). Since hippocampus and prefrontal lobe are two regions that are significantly affected in emotional and cognitive disorders²⁵, we studied alterations in RPTN expression in these regions in mice subjected to the CUMS procedure, a widely used animal model for the study of major depression¹⁸. Over the 21-day period of CUMS

procedure, RPTN protein levels in hippocampus and prefrontal cortex gradually declined, as determined by Western blot (Figure 2-d).

Serum RPTN levels in normal human subjects, schizophrenia, and bipolar disorder. Given that the detection limit for the human RPTN kit is 156 pg/ml, all serum samples with RPTN level below 156 pg/ml were assigned as RPTN undetectable. Then, the median of each group was used to indicate the average level of serum RPTN. Kruskal-Wallis test was used to analyze the data (Table 2). In normal human subjects, the median serum RPTN is 915.0 pg/ml ($n = 115$), but some samples had levels as high as 10,000 pg/ml serum RPTN. Of 88 schizophrenia serum samples, the median RPTN concentration is 156.0 pg/ml; of these only two samples showed detectable levels of 163 pg/ml and 309 pg/ml, whereas most patient sera (97.7%) showed an undetectable level of RPTN. In 34 bipolar disorder patients, serum RPTN had a median of 156.0 pg/ml; three samples had 165, 303 and 308 pg/ml RPTN; all the rest of the samples (91.2%) were RPTN undetectable with the current ELISA method. The difference of serum RPTN levels between healthy controls and the patients was extremely significant ($p < 0.0001$) (Figure 3-a). Since almost all patients had nearly undetectable levels of RPTN, medication use or duration of illness were thus unlikely to be correlative factors to RPTN levels. There was no significant difference in serum RPTN levels between schizophrenia and bipolar disorder. In normal human subjects, serum RPTN levels among the age groups or between male and female were not significantly different (Figure 3-b, c).

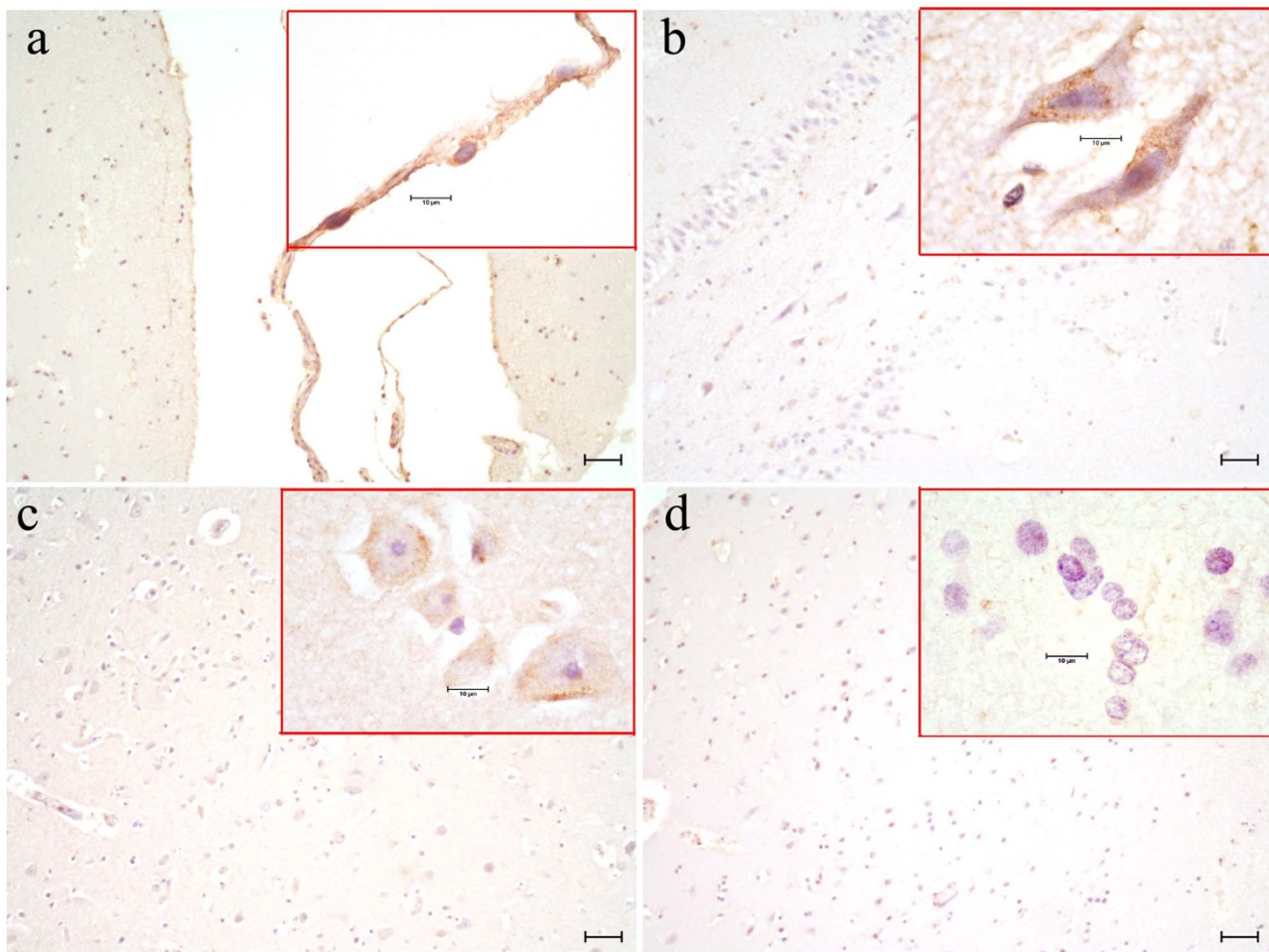


Figure 1 | Immunohistochemical detection of RPTN expression in normal human brain. a, choroid plexus (ependyma). b, hippocampus. c, prefrontal lobe. d, temporal lobe. The inserts are high-power images of selected regions. Scale bars are 50 μ m. Scale bars in the inserts are 10 μ m.

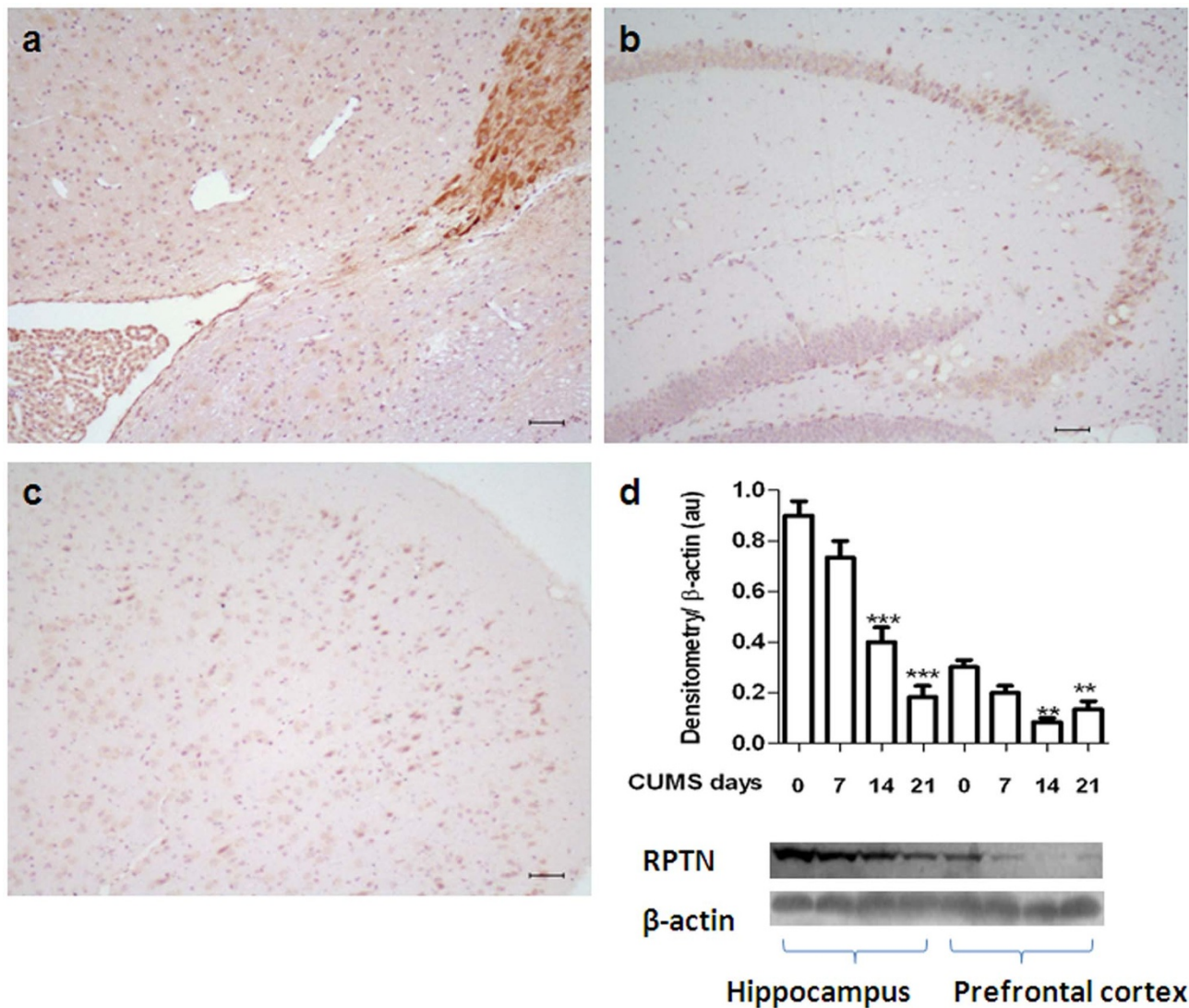


Figure 2 | Immunohistochemistry and Western blot detection of RPTN expression in mouse brain. a, Choroid plexus (fourth ventricle), ependyma and locus coeruleus. b, hippocampus. c, prefrontal cortex. d, lower panels are Western Blot of RPTN and endogenous β -actin control in the hippocampus and prefrontal cortex of mice subjected to CUMS on day 0 to 21; upper graph is the densitometry analyses of RPTN over corresponding β -actin and expressed as arbitrary units (au) ($n = 4$). Scale bars are 50 μ m. ** $p < 0.05$, *** $p < 0.0001$.

Serum RPTN levels in drug users and age-matched normal human subjects. The median serum RPTN of drug users ($n = 91$) was 156.0 pg/ml (156.0 at 25% percentile and 738.0 at 75% percentile),

compared to 1034 pg/ml (352.8 at 25% percentile and 2518 at 75% percentile) in age-matched normal subjects ($n = 92$). The drug users showed a significant reduction of serum RPTN compared to age-

Table 2 | Statistical analyses (Kruskal-Wallis multiple comparison test)

Serum RPTN (pg/ml)	Sample size	Median	25% percentile	75% percentile	P Value#
Human					
Healthy controls	115	915.0	364.0	2003.0	
Schizophrenia	88	156.0	156.0	156.0	<0.0001
Bipolar disorder	34	156.0	156.0	156.0	<0.0001
Age-matched controls	92	1034.0	352.8	2518.0	
Methamphetamine users	91	156.0	156.0	738.0	<0.0001##
Animals					
Normal mice	11	340.0	240.0	608.0	
CUMS mice	16	494.0	318.5	648.8	ns
Olanzapine treatment	9	924.0	770.0	1236.0	<0.0001
Quetiapine treatment	7	800.0	770.0	1080.0	<0.05
Aripiprazole treatment	9	609.0	379.5	905.5	ns

#: compared with controls of human or mice; ##: compared with age-matched controls; ns: no significance.

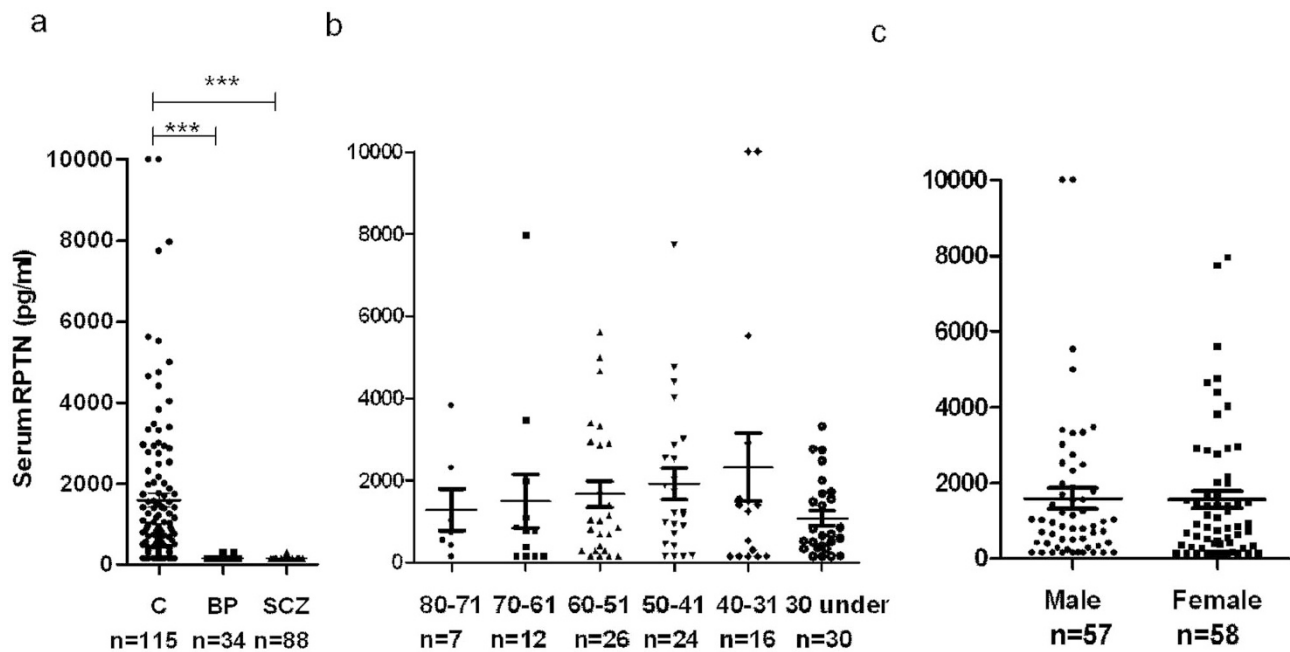


Figure 3 | Serum RPTN levels in control and patients with schizophrenia (SCZ) or bipolar disorder (BP). a, serum RPTN levels in control (C), SCZ and BP. b, serum RPTN levels in different age groups of control, from 30 years and under to 71–80 year group. c, serum RPTN in males and females of control. *** $p < 0.0001$.

matched healthy human subjects ($p < 0.0001$), but a significant elevation of RPTN relative to the schizophrenia group ($p < 0.0001$) (Table 2 and Figure 4-a).

Serum RPTN levels in normal mice, CUMS model mice and drug treated mice. Since the majority of the patients enrolled in the study were under atypical antipsychotic therapy, to rule out the possibility that the reduction of serum RPTN was due to antipsychotics, we tested the effect of three widely used drugs on serum RPTN levels in mice. Normal mice had a median serum RPTN of 340.0 pg/ml; as compared to 924.0, 800.0 or 609.0 pg/ml in mice treated with Olanzapine, Quetiapine or Aripiprazole, respectively. Statistical analysis indicated no effect on serum RPTN level from Aripiprazole treatment, but there was a significant increase in serum RPTN from Olanzapine or Quetiapine treatment. However, the results did not show a significant difference between CUMS (494.0 pg/ml) mice and normal mice in serum RPTN levels.

To confirm the reliability of the ELISA kit used in the study, a recovery experiment was performed with both low (300 pg/ml) and high (2500 pg/ml) RPTN concentrations. The recovery rates of low and high concentrations of RPTN were $104 \pm 16.9\%$ and $102 \pm 13.5\%$, respectively ($n = 8$).

Discussion

RPTN was previously only known to be expressed in normal epidermis; this study is the first time that RPTN expression in brain has been reported.

Our primary finding is that serum RPTN is significantly diminished in patients with schizophrenia and bipolar disorder. The results obtained in mice treated with antipsychotics for 24 days may not rule out completely the possibility that the reduction of serum RPTN in the patients is the result of multi-year antipsychotic therapy, but this confounding possibility would not be applicable for comparison to patients under acute antipsychotic medication. In contrast, atypical antipsychotics actually increase the serum level of RPTN in mice. Similarly, S100B is increased in schizophrenics who are being treated with antipsychotics²⁶. Since almost all patients had nearly undetectable levels of RPTN, no correlation between disease severity and

RPTN concentration in serum is suggested, which is also quite similar to the reported role of S100B in schizophrenia^{13,14}. It is noteworthy that a few normal subjects also had undetectable RPTN levels. The low levels of RPTN in the normal subjects may be due to the limited sensitivity of the detection kit used in this study. A more sensitive detection method may differentiate the healthy subjects with low RPTN levels from the psychiatric patients. Meanwhile, we cannot rule out the possibility that those normal subjects with undetectable serum RPTN may be inflicted with minor covert emotional or cognitive disorders. From the results of the CUMS mouse model in this study, stress had such a significant effect on RPTN expression that a significant decrease appeared in as little as one week, in both hippocampus and prefrontal cortex (Figure 2-d).

Due to the lack of postmortem samples, this study was only able to analyze RPTN levels on a normal human brain chip. We have not found out any relevant information about RPTN alterations in available microarray data such as a transcriptome of postmortem brains of schizophrenics²⁷, proteomic analyses of schizophrenia hippocampus²⁸ and gene expression profiles of dorsolateral prefrontal cortex of schizophrenia patients²⁹. Changes in S100B have been frequently identified in schizophrenia, but it is not found in these databases either. Although the reduction of RPTN in CUMS mouse brain may not suggest a role in the reduction of serum RPTN in schizophrenia, there is evidence that depression and schizophrenia often affect the same brain regions^{30–33}. To confirm further the alteration of serum RPTN in patients with schizophrenia, we analyzed the serum RPTN levels in poly-drug users. A nation-wide survey of drug abuse in China showed most heroin users have used METH frequently³⁴. The 91 poly-drug users in this study have used both heroin and METH for more than 3 years. The prevalence of comorbid psychiatric disorders among drug users under methadone maintenance treatment is almost six times higher than that of normal subjects³⁵. Chronic heroin use could reduce serum neurotrophins such as brain-derived neurotrophic factor (BDNF) and thus may increase the risk of developing psychosis³⁶, while chronic METH abuse induced psychosis recapitulates many of the psychotic symptoms of schizophrenia³⁷. The reduction of serum RPTN in poly-drug users may thus reflect its decrease in schizophrenia. RPTN expression is

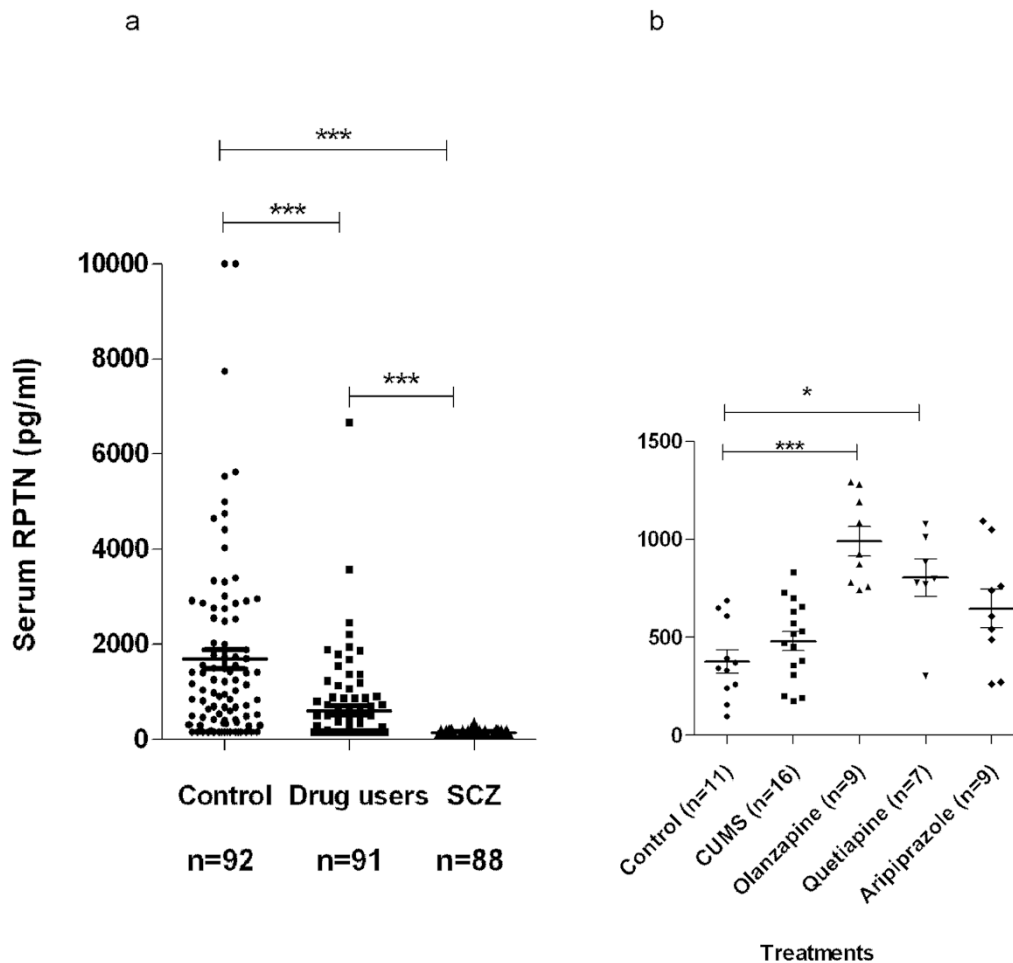


Figure 4 | Serum RPTN levels in human subjects of drug users, age-matched healthy controls and schizophrenia (SCZ) and in normal and CUMS mice and mice treated with atypical antipsychotics. a, serum RPTN levels in drug users and age-matched healthy controls and schizophrenic patients. b, serum RPTN levels in normal and CUMS mice and mice treated with Olanzapine, Quetiapine or Aripiprazole for 24 days. * $p < 0.05$, *** $p < 0.0001$.

reduced in hippocampi and prefrontal lobes of CUMS mice, but the serum RPTN level remains unchanged. The different patterns of expression of RPTN between CUMS mice and schizophrenic patients may imply its universal role in the pathogenesis of neuropsychiatric disorders. Whether the alteration of RPTN is a contributor to, or is a consequence of the diseases remains to be elucidated. A future study on postmortem brain or *in vivo* examination via imaging techniques may help elucidate the mechanisms involved.

The origin of serum RPTN is currently unclear. In this study, serum RPTN levels are increased in mice treated with atypical antipsychotics, but decreased in poly-drug users. Given that METH can stimulate the release of serotonin (5HT), dopamine (DA) and norepinephrine (NE)³⁸ while atypical antipsychotics act as antagonists of 5HT/D2, this may imply that serum RPTN is regulated by the DA neurotransmitter system.

RPTN functions in the cornified cell envelope formation¹⁷, but its role in the CNS remains unknown. RPTN protein contains two EF-hands, which structurally resemble the calcium binding domain of PV¹⁷. Calcium-binding proteins play important roles in the brain and in psychiatric disorders. PV is regarded as a neuronal marker and has a potential key role in the pathophysiology of schizophrenia³⁹, as its reduction in ventral hippocampus can induce an augmented DA system function and behavioral hyper-responsivity to amphetamine⁶. The calcium-binding proteins calbindin (CB), calretinin (CR) and PV are considered as markers of specific subpopulations of cortical GABAergic interneurons. The densities of CB-immunoreactive neurons in layer 2 and PV-immunoreactive neurons in layer 4

in schizophrenia subjects are decreased⁴⁰. S100B is another calcium-binding protein that is increased significantly in the sera of schizophrenic patients⁴¹; its elevation in the CSF may indicate dysfunction of glial cells and the blood-brain barrier in schizophrenia^{11,42}.

The hippocampus and prefrontal cortex are strongly implicated in depression, bipolar disorder and schizophrenia^{1,5,43–45}. Postmortem and brain imaging studies of depressed and anxious patients show that chronic stress can affect prefrontal cortex and hippocampus^{30,31}. Presynaptic abnormalities and prefrontal cortical dysfunction were reported to exist in schizophrenia⁴⁶. Deficits in functional integration between prefrontal cortex and hippocampus, or their dysfunctions, have been associated with cognitive impairment in schizophrenia^{32,33}. RNA-Seq data from the hippocampus has indicated that abnormal immune/inflammation response in the hippocampus may underlie the pathophysiology of schizophrenia and may be associated with abnormalities in the PV-containing neurons that lead to the cognitive deficits of the disease⁴⁷. In the hippocampus, the CA1 pyramidal layer shows bilaterally decreased S100B-immunopositive astrocytes in major depressive disorder and bipolar disorder patients¹⁰. CA2 and CA3 regions were affected to a significantly greater degree than other hippocampal regions in patients with schizophrenia and bipolar disorder²⁸. In this study, the relatively high levels of RPTN in CA3 and CA2 regions of the hippocampus and prefrontal lobe (figure 1,b-c; figure 2,b-c) may suggest a role of RPTN in the process of emotional and cognitive regulation. Accordingly, the reduced expression of RPTN in hippocampus and prefrontal cortex of the CUMS mouse (figure 2-d) may further imply its involvement in depression, schizophrenia and bipolar disorder.



In this study, RPTN showed high expression levels in the choroid plexus and ependyma (figure 1-a, figure 2-a). A previous study found that an epidermal calcium-binding protein was detected in the ependyma of brain⁴⁸. Schizophrenia may result from covert immune complex disease of the basal lamina of the choroid plexus, which is similar to the structures of skin⁴⁹. The presence of choroid plexus calcification (CPC) is associated with hippocampal, frontal, temporoparietal and cerebellar atrophies. CPC may be a predictive indicator of poor evolution or of a neurodegenerative type⁵⁰, a neuroradiological marker of hallucinations⁵¹ or depression in schizophrenia⁵². There is also a possible correlation between CPC and dysgenetic or functional 5HT alteration⁵³. RPTN is a component of the skin barrier^{54,55}, so its presence in choroid plexus and ependymal cells may suggest its role in maintaining the integrity of blood-CSF barrier and associated functions.

Interestingly, RPTN showed the highest expression level in the LC in mouse brain (figure 2-a). In the human brain chip, however, LC was not identified, because the chip contained no LC region. LC has been proposed to be involved in the pathophysiology of aging and schizophrenia, since its volume is reduced in schizophrenic brains⁵⁶, but DA-beta-hydroxylase activity was increased in the rostral part⁵⁷. A neuromelanin-sensitive magnetic resonance imaging study found that the contrast ratio of LC to the adjacent white matter in depressive patients was significantly lower than that of control subjects and schizophrenic patients⁵⁸. The LC is the principal site for brain synthesis of NE. Catecholamine neurons are considered as the main target of METH since METH is a powerful DA and NE releaser⁵⁹. Therefore, serum RPTN reduction in drug users may imply its involvement in the LC-noradrenergic system.

Collectively, being a calcium-binding protein expressed in crucial regions in brain, RPTN may have roles in calcium homeostasis or/and calcium signaling. Since dysregulation of the calcium-signaling pathway has been implicated in the development of bipolar disorder and schizophrenia^{3,4}, the diminished serum RPTN observed in the patients suggests its possible involvement in the pathogenesis of those disorders.

1. Sawa, A. & Kamiya, A. Elucidating the pathogenesis of schizophrenia. *Bmj* **327**, 632–633 (2003).
2. Lichtenstein, P. *et al.* Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. *Lancet* **373**, 234–239 (2009).
3. Ripke, S. *et al.* Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nat Genet* **45**, 1150–1159 (2013).
4. Berridge, M. J. Calcium signalling and psychiatric disease: bipolar disorder and schizophrenia. *Cell Tissue Res* **357**, 477–492 (2014).
5. Glausier, J. R., Fish, K. N. & Lewis, D. A. Altered parvalbumin basket cell inputs in the dorsolateral prefrontal cortex of schizophrenia subjects. *Mol Psychiatry* **19**, 30–36 (2014).
6. Boley, A. M., Perez, S. M. & Lodge, D. J. A fundamental role for hippocampal parvalbumin in the dopamine hyperfunction associated with schizophrenia. *Schizophr Res* **157**, 238–243 (2014).
7. Wang, A. Y. *et al.* Bipolar disorder type 1 and schizophrenia are accompanied by decreased density of parvalbumin- and somatostatin-positive interneurons in the parahippocampal region. *Acta Neuropathol* **122**, 615–626 (2011).
8. Konradi, C. *et al.* Hippocampal interneurons are abnormal in schizophrenia. *Schizophr Res* **131**, 165–173 (2011).
9. Yelmo-Cruz, S., Morera-Fumero, A. L. & Abreu-Gonzalez, P. S100B and schizophrenia. *Psychiatry Clin Neurosci* **67**, 67–75 (2013).
10. Gos, T. *et al.* S100B-immunopositive astrocytes and oligodendrocytes in the hippocampus are differentially afflicted in unipolar and bipolar depression: a postmortem study. *J Psychiatr Res* **47**, 1694–1699 (2013).
11. Rothermundt, M. *et al.* Glial cell dysfunction in schizophrenia indicated by increased S100B in the CSF. *Mol Psychiatry* **9**, 897–899 (2004).
12. Lambert, J. C. *et al.* Evidence for the association of the S100beta gene with low cognitive performance and dementia in the elderly. *Mol Psychiatry* **12**, 870–880 (2007).
13. Schroeter, M. L. & Steiner, J. Elevated serum levels of the glial marker protein S100B are not specific for schizophrenia or mood disorders. *Mol Psychiatry* **14**, 235–237 (2009).
14. Steiner, J., Bogerts, B., Schroeter, M. L. & Bernstein, H. G. S100B protein in neurodegenerative disorders. *Clin Chem Lab Med* **49**, 409–424 (2011).
15. van der Leeuw, C. *et al.* Replicated evidence of absence of association between serum S100B and (risk of) psychotic disorder. *PLoS One* **8**, e82535 (2013).
16. Krieg, P. *et al.* Repetin (Rptn), a new member of the "fused gene" subgroup within the S100 gene family encoding a murine epidermal differentiation protein. *Genomics* **43**, 339–348 (1997).
17. Huber, M. *et al.* Isolation and characterization of human repetin, a member of the fused gene family of the epidermal differentiation complex. *J Invest Dermatol* **124**, 998–1007 (2005).
18. Nollet, M., Le Guisquet, A. M. & Belzung, C. Models of depression: unpredictable chronic mild stress in mice. *Curr Protoc Pharmacol* **Chapter 5**, Unit 5 65 (2013).
19. Qiao, H., An, S. C., Ren, W. & Ma, X. M. Progressive alterations of hippocampal CA3-CA1 synapses in an animal model of depression. *Behav Brain Res* **275C**, 191–200 (2014).
20. Imai, S. *et al.* Ubiquitin-specific peptidase 46 (Usp46) regulates mouse immobile behavior in the tail suspension test through the GABAergic system. *PLoS One* **7**, e39084 (2012).
21. Cryan, J. F. & Mombereau, C. In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. *Mol Psychiatry* **9**, 326–357 (2004).
22. Ferno, J. *et al.* Olanzapine-induced hyperphagia and weight gain associate with orexigenic hypothalamic neuropeptide signaling without concomitant AMPK phosphorylation. *PLoS One* **6**, e20571 (2011).
23. He, J., Kong, J., Tan, Q. R. & Li, X. M. Neuroprotective effect of atypical antipsychotics in cognitive and non-cognitive behavioral impairment in animal models. *Cell Adh Migr* **3**, 129–137 (2009).
24. Picada, J. N. *et al.* Neurobehavioral and genotoxic parameters of antipsychotic agent aripiprazole in mice. *Acta Pharmacol Sin* **32**, 1225–1232 (2011).
25. Vertes, R. P. Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. *Neuroscience* **142**, 1–20 (2006).
26. Schroeter, M. L. *et al.* Serum S100B is increased during early treatment with antipsychotics and in deficit schizophrenia. *Schizophr Res* **62**, 231–236 (2003).
27. Roussos, P., Katsel, P., Davis, K. L., Siever, L. J. & Haroutunian, V. A system-level transcriptomic analysis of schizophrenia using postmortem brain tissue samples. *Arch Gen Psychiatry* **69**, 1205–1213 (2012).
28. Focking, M. *et al.* Common proteomic changes in the hippocampus in schizophrenia and bipolar disorder and particular evidence for involvement of cornu ammonis regions 2 and 3. *Arch Gen Psychiatry* **68**, 477–488 (2011).
29. Guillozet-Bongaarts, A. L. *et al.* Altered gene expression in the dorsolateral prefrontal cortex of individuals with schizophrenia. *Mol Psychiatry* **19**, 478–485 (2014).
30. Shah, P. J., Ebmeier, K. P., Glabus, M. F. & Goodwin, G. M. Cortical grey matter reductions associated with treatment-resistant chronic unipolar depression. Controlled magnetic resonance imaging study. *Br J Psychiatry* **172**, 527–532 (1998).
31. Sheline, Y. I., Wang, P. W., Gado, M. H., Csernansky, J. G. & Vannier, M. W. Hippocampal atrophy in recurrent major depression. *Proc Natl Acad Sci USA* **93**, 3908–3913 (1996).
32. Zierhut, K. *et al.* The role of hippocampus dysfunction in deficient memory encoding and positive symptoms in schizophrenia. *Psychiatry Res* **183**, 187–194 (2010).
33. Benetti, S. *et al.* Functional integration between the posterior hippocampus and prefrontal cortex is impaired in both first episode schizophrenia and the at risk mental state. *Brain* **132**, 2426–2436 (2009).
34. Sun, H. Q., Bao, Y. P., Zhou, S. J., Meng, S. Q. & Lu, L. The new pattern of drug abuse in China. *Curr Opin Psychiatry* **27**, 251–255 (2014).
35. Fan, C. Y., Tan, H. K., Chien, I. C. & Chou, S. Y. Prevalence of psychiatric disorders among heroin users who received methadone maintenance therapy in Taiwan. *Am J Addict* **23**, 249–256 (2014).
36. Angelucci, F. *et al.* Chronic heroin and cocaine abuse is associated with decreased serum concentrations of the nerve growth factor and brain-derived neurotrophic factor. *J Psychopharmacol* **21**, 820–825 (2007).
37. Hsieh, J. H., Stein, D. J. & Howells, F. M. The neurobiology of methamphetamine induced psychosis. *Front Hum Neurosci* **8**, 537 (2014).
38. Rothman, R. B. *et al.* Neurochemical neutralization of methamphetamine with high-affinity nonselective inhibitors of biogenic amine transporters: a pharmacological strategy for treating stimulant abuse. *Synapse* **35**, 222–227 (2000).
39. Celio, M. R. & Heizmann, C. W. Calcium-binding protein parvalbumin as a neuronal marker. *Nature* **293**, 300–302 (1981).
40. Sakai, T. *et al.* Changes in density of calcium-binding-protein-immunoreactive GABAergic neurons in prefrontal cortex in schizophrenia and bipolar disorder. *Neuropathology* **28**, 143–150 (2008).
41. Rothermundt, M. *et al.* Increased S100B blood levels in unmedicated and treated schizophrenic patients are correlated with negative symptomatology. *Mol Psychiatry* **6**, 445–449 (2001).
42. Schroeter, M. L., Abdul-Khalik, H., Krebs, M., Diefenbacher, A. & Blasig, I. E. Neuron-specific enolase is unaltered whereas S100B is elevated in serum of patients with schizophrenia—original research and meta-analysis. *Psychiatry Res* **167**, 66–72 (2009).



43. Kim, S. & Webster, M. J. Integrative genome-wide association analysis of cytoarchitectural abnormalities in the prefrontal cortex of psychiatric disorders. *Mol Psychiatry* **16**, 452–461 (2011).
44. Ewing, S. G. & Winter, C. The ventral portion of the CA1 region of the hippocampus and the prefrontal cortex as candidate regions for neuromodulation in schizophrenia. *Med Hypotheses* **80**, 827–832 (2013).
45. Heckers, S. The hippocampus in schizophrenia. *Am J Psychiatry* **161**, 2138–2139 (2004).
46. Porton, B. & Wetsel, W. C. Reduction of synapsin III in the prefrontal cortex of individuals with schizophrenia. *Schizophr Res* **94**, 366–370 (2007).
47. Hwang, Y. *et al.* Gene expression profiling by mRNA sequencing reveals increased expression of immune/inflammation-related genes in the hippocampus of individuals with schizophrenia. *Transl Psychiatry* **3**, e321 (2013).
48. Pavlovitch, J. H., Didierjean, L., Rizk, M., Balsan, S. & Saurat, J. H. Skin calcium-binding protein: distribution in other tissues. *Am J Physiol* **244**, C50–57 (1983).
49. Rudin, D. O. Covert transport dysfunction in the choroid plexus as a possible cause of schizophrenia. *Schizophr Bull* **5**, 623–626 (1979).
50. Marinescu, I., Udristoiu, I. & Marinescu, D. Choroid plexus calcification: clinical, neuroimaging and histopathological correlations in schizophrenia. *Rom J Morphol Embryol* **54**, 365–369 (2013).
51. Sandyk, R. Choroid plexus calcification as a possible marker of hallucinations in schizophrenia. *Int J Neurosci* **71**, 87–92 (1993).
52. Sandyk, R., Kay, S. R. & Merriam, A. E. Calcification of the choroid plexus as a marker of depression in schizophrenia. *Schizophr Res* **3**, 361–363 (1990).
53. Bersani, G., Garavini, A., Taddei, I., Tanfani, G. & Pancheri, P. Choroid plexus calcification as a possible clue of serotonin implication in schizophrenia. *Neurosci Lett* **259**, 169–172 (1999).
54. Koch, P. J. *et al.* Lessons from loricrin-deficient mice: compensatory mechanisms maintaining skin barrier function in the absence of a major cornified envelope protein. *J Cell Biol* **151**, 389–400 (2000).
55. Segre, J. A., Bauer, C. & Fuchs, E. Klf4 is a transcription factor required for establishing the barrier function of the skin. *Nat Genet* **22**, 356–360 (1999).
56. Lohr, J. B. & Jeste, D. V. Locus ceruleus morphometry in aging and schizophrenia. *Acta Psychiatr Scand* **77**, 689–697 (1988).
57. Gay, N., Cottiaux, J. A., Denoroy, L., Tommasi, M. & Kopp, N. Possible increase of dopamine-beta-hydroxylase activity in the locus ceruleus of paranoid schizophrenic patients: a preliminary post-mortem study. *Psychiatry Res* **27**, 31–38 (1989).
58. Shibata, E. *et al.* Use of neuromelanin-sensitive MRI to distinguish schizophrenic and depressive patients and healthy individuals based on signal alterations in the substantia nigra and locus ceruleus. *Biol Psychiatry* **64**, 401–406 (2008).
59. Ferrucci, M., Giorgi, F. S., Bartalucci, A., Busceti, C. L. & Fornai, F. The effects of locus coeruleus and norepinephrine in methamphetamine toxicity. *Curr Neuropharmacol* **11**, 80–94 (2013).

Acknowledgments

This work was supported by National Natural Science Foundation of China (31170855, 81272257) and National Basic Research Program of China (973) (2012CB720604).

Author contributions

H.R., Y.Y. and L.Z. performed ELISA assay; S.W. and S.H. performed western blot and IHC; J.X., T.W., H.Q., C.X., B.J., C.Y. and S.A. generated CUMS mice and performed validations; C.W. and S.C. prepared figures; L.Z., Z.Y. and E.W.T. wrote the manuscript. All authors reviewed the manuscript.

Additional information

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Wang, S. *et al.* Diminished serum repetin levels in patients with schizophrenia and bipolar disorder. *Sci. Rep.* **5**, 7977; DOI:10.1038/srep07977 (2015).



This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder in order to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-sa/4.0/>